

WHAT IS CLAIMED IS:

A method for identifying an organic molecule ligand that binds to a site of interest on a biological target molecule, said method comprising:

- 5 (a) obtaining a biological target molecule that comprises or has been modified to comprise a chemically reactive group, wherein said site of interest on said target molecule comprises said chemically reactive group;
- 10 (b) combining said target molecule with one or more members of a library of organic compounds that are capable of covalently bonding to said chemically reactive group, wherein at least one member of said library binds to said site of interest and forms a covalent bond with said chemically reactive group to form a target molecule/organic compound conjugate; and
- 15 (c) identifying the organic compound that forms a covalent bond with said chemically reactive group.

- 20 2. The method according to Claim 1, wherein said biological target molecule is selected from the group consisting of a polypeptide, a nucleic acid, a carbohydrate, a nucleoprotein, a glycopeptide, a glycolipid and a lipoprotein.

3. The method according to Claim 2, wherein said biological target molecule is a polypeptide.

4. The method according to Claim 3, wherein said polypeptide is selected from the group consisting of an enzyme, a hormone, a transcription factor, a receptor, a ligand for a receptor, a growth factor
- 25 and an immunoglobulin.

5. The method according to Claim 1, wherein said biological target molecule comprises said chemically reactive group without prior modification of said target molecule.

6. The method according to Claim 1, wherein said biological target molecule obtained in step (a) has been modified to comprise said chemically reactive group.

7. The method according to Claim 6, wherein said modification comprises bonding to said target molecule a compound that comprises said chemically reactive group.

8. The method according to Claim 1, wherein said library of organic compounds comprises aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, thioesters, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds and acid chlorides.

9. The method according to Claim 1, wherein said library of organic compounds comprises primary amines, secondary amines, aldehydes or ketones.

10. The method according to Claim 1, wherein said chemically reactive group is a primary amine group, a secondary amine group, an aldehyde group or a ketone group.

11. The method according to Claim 1, wherein step (c) is accomplished by a process that employs mass spectrometry.

12. The method according to Claim 1, wherein step (c) comprises fragmenting said target molecule/organic compound conjugate
5 into two or more fragments.

13. The method according to Claim 1, wherein subsequent to step (b) and prior to step (c) said target molecule/organic compound conjugate is combined with one or more members of a library of organic molecules that are capable of covalently bonding to the organic
10 compound previously bound to said target molecule, wherein at least one member of said library of organic molecules binds to said target molecule/organic compound conjugate.

14. A method for identifying an organic molecule ligand that binds to a biological target molecule of interest, said method comprising:
15 (a) obtaining a biological target molecule that comprises or has been modified to comprise a first reactive functionality,
(b) reacting said target molecule with a compound that comprises (1) a second reactive functionality and (2) a chemically reactive group, wherein said second reactive functionality reacts with said first
20 reactive functionality of said target molecule to form a covalent bond, thereby resulting in said chemically reactive group being linked to said target molecule through a covalent bond;
(c) combining said target molecule with one or more members of a library of organic compounds that are capable of covalently bonding to
25 said chemically reactive group, wherein at least one member of said library forms a covalent bond with said chemically reactive group to form a target molecule/organic compound conjugate; and
(d) identifying the organic compound that forms a covalent bond with said chemically reactive group.

15. The method according to Claim 14, wherein said first and second chemically reactive functionalities are activated thiol groups that react to form a disulfide bond.

16. The method according to Claim 15, which further comprises
5 subsequent to step (c) and prior to step (d) the step of liberating the covalently-bonded organic compound from said target molecule/organic compound conjugate by treatment with an agent that disrupts said disulfide bond.

17. The method according to Claim 16, wherein said agent that
10 disrupts said disulfide bond is dithiothreitol, dithioerythritol, β -mercaptoethanol, sodium borohydride or a phosphine.

18. The method according to Claim 14, wherein said biological target molecule is selected from the group consisting of a polypeptide, a nucleic acid, a carbohydrate, a nucleoprotein, a glycopeptide, a glycolipid
15 and a lipoprotein.

19. The method according to Claim 18, wherein said biological target molecule is a polypeptide.

20. The method according to Claim 19, wherein said polypeptide is selected from the group consisting of an enzyme, a hormone, a
20 transcription factor, a receptor, a ligand for a receptor, a growth factor and an immunoglobulin.

21. The method according to Claim 19, wherein said polypeptide comprises or has been modified to comprise only a single cysteine residue.

22. The method according to Claim 19, wherein said polypeptide is obtained as a recombinant expression product.

23. The method according to Claim 19, wherein said polypeptide is synthetically derived.

5 24. The method according to Claim 14, wherein said target molecule comprises or has been modified to comprise less than about 2 free thiol groups.

25. The method according to Claim 14, wherein said library of organic compounds comprises aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, thioesters, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic
10 compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds and acid chlorides.
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26. The method according to Claim 14, wherein said chemically
20 reactive group is selected from the group consisting of an aldehyde group and a ketone group and said library of organic compounds comprises primary amines and/or secondary amines.

27. The method according to Claim 14, wherein said chemically
25 reactive group is selected from the group consisting of a primary amine group and a secondary amine group and said library of organic compounds comprises aldehydes and/or ketones.

28. The method according to Claim 14, wherein in step (c) one member of said library of organic compounds reacts with said chemically reactive group to form a Schiff base adduct.

29. The method according to Claim 28, wherein subsequent to step (c) and prior to step (d), said Schiff base adduct is reduced by addition of a reducing agent.

30. The method according to Claim 29, wherein said reducing agent is selected from the group consisting of sodium cyanoborohydride, sodium triacetoxyborohydride and cyanide.

31. The method according to Claim 14, wherein said step (d) is accomplished by a process that employs mass spectrometry.

32. A method for identifying a ligand that binds to a biological target molecule of interest, said method comprising:

(a) identifying a first organic molecule ligand that binds to said biological target molecule by the method of Claim 1;

(b) identifying a second organic molecule ligand that binds to said biological target molecule by the method of Claim 1; and

(c) linking said first and second organic molecule ligands through a linker element to form a conjugate molecule that binds to said biological target molecule.

33. The method according to Claim 32, wherein said biological target molecule is selected from the group consisting of a polypeptide, a nucleic acid, a carbohydrate, a nucleoprotein, a glycopeptide, a glycolipid and a lipoprotein.

34. The method according to Claim 32, wherein said biological target molecule is a polypeptide.

35. The method according to Claim 34, wherein said first and said second organic molecule ligands bind to the same site on said polypeptide.

36. The method according to Claim 34, wherein said first and
5 said second organic molecule ligands bind to different sites on said polypeptide.

37. The method according to Claim 32, wherein said first and second organic molecule ligands are from the same chemical class.

38. The method according to Claim 32, wherein said first and
10 second organic molecule ligands are from different chemical classes.

39. The method according to Claim 34, wherein said conjugate molecule binds to said polypeptide with a lower dissociation constant than either of said first and second organic molecule ligands.

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